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Diet fat influences liver plasma-membrane lipid composition and glucagon-stimulated adenylate cyclase activity

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Rats were fed diets that differed in fatty acid composition or in the proportion of energy derived from fat to determine if alteration of dietary fat intake influences the structural lipid composition of liver plasma membrane and the expression of an associated hormone-receptor-mediated function. Weanling rats were fed 9% (w/w) or 20% (w/w) low-erucic acid rape-seed oil or 9% (w/w) soya-bean oil for 24 days. Plasma membranes were isolated and the effect of diet fat on the fatty acid composition of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol and sphingomyelin was determined. Diet fat significantly altered total saturated and ($\omega-9$) and ($\omega-6$)-unsaturated fatty acid composition in addition to the ($\omega-6$)- to ($\omega-3$)-unsaturated fatty acid ratio in these polar lipids. Feeding the high-fat diet increased the ($\omega-6$)- to ($\omega-3$)-unsaturated fatty acid ratio and the ($\omega-9$)-unsaturated fatty acid content in all lipids except sphingomyelin. Assay of glucagon-stimulated adenylate cyclase activity at both high and low glucagon concentrations indicated that high-fat intake also decreased cyclic AMP formation. In a second experiment the fat intake was held constant (40% of energy) and oleic acid was substituted for linoleic acid by blending high- and low-linoleic acid-type safflower oils. This experiment established that a dose–response relationship exists between dietary intake of fatty acid and the fatty acid composition of plasma-membrane phospholipids. Specific diet-induced transitions in membrane phospholipid fatty acid composition were paralleled by changes in glucagon-stimulated adenylate cyclase activity. This study suggests that transitions in dietary fat intake can alter a hormone-receptor-mediated enzyme function *in vivo* by changing the surrounding lipid environment.

The currently accepted model of membrane structure (Singer & Nicholson, 1972) proposes a dynamic, asymmetric lipid matrix of phospholipids and cholesterol with globular proteins embedded across the membrane to various degrees. Most phospholipids are in the bilayer arrangement and may also be closely associated with integral membrane proteins or loosely associated with peripheral proteins. Biological functions of membranes, such as membrane-bound enzyme functions and transport systems, are influenced by the membrane physical properties, which are determined by fatty acid composition of polar lipids, polar head group composition and membrane cholesterol content (Holub & Kuksis, 1978). Polar and non-polar regions of the phospholipid molecule may interact, with changes in the conformation of a membrane-associated protein altering either its catalytic activity or the protein's interaction with other membrane proteins (Coleman, 1973).

The liver plasma membrane plays an important role in maintaining whole-body homoeostasis, acting as an interface between circulating hormones and hormone-activated functions within the hepatocyte. Alteration of the structural composition of this plasma membrane may influence the physical properties of the membrane and this may alter hormone-receptor-mediated functions. In this regard, liver plasma membrane lipid composition can be altered in rodents by diets deficient in essential fatty acids (Sun *et al.*, 1979). However, it is not known whether alteration in dietary lipid intake can alter the structural constituents of this membrane when a nutritionally adequate diet is fed.

Glucagon can be regarded as a suitable model for a polypeptide hormone that exerts its effect on subcellular metabolism via a receptor by activating a specific enzyme function in the target cell membrane. Glucagon stimulates adenylate cyclase in the plasma membrane, increasing intracellular concentrations of cyclic AMP. Glucagon-activated adenylate cyclase consists of three mobile components: a receptor unit that interacts with the hormone, a catalytic unit that carries out the conversion of ATP into cyclic AMP; and the transducer component that links the receptor to the catalytic unit (Houslay *et al.*, 1977). GTP is also required for hormonal activation of adenylate cyclase (Kimura & Nagata, 1977; Levitzki & Helmreich, 1979). Phospholipid is essential for hormone-stimulated adenylate cyclase activity, but the site of involvement is not clear. Both a non-specific requirement for membrane fluidity to allow for interaction between hormone–receptor and catalytic components (Dipple & Houslay, 1978) and a specific requirement for phosphatidylserine at the level of hormone binding have been proposed (Rethy *et al.*, 1972; Rubakcava & Rodbell, 1973; Birnbaumer, 1973). In addition, it is known that glucagon-stimulated adenylate cyclase activity is lower in liver plasma membranes of rats fed essential-fatty-acid-deficient diets (Louis *et al.*, 1976).

Two experiments were designed to assess the influence of nutritionally complete diets differing in fatty acid content on fatty acyl tail composition of liver plasma-membrane phospholipids and on glucagon-stimulated adenylate cyclase activity. In the first experiment diet fat was found to alter fatty acyl tail composition of plasma-membrane phospholipids and influence activity of this hormone-stimulated plasma-membrane-associated function. By dietary manipulation, the second experiment established that a dose–response relationship exists between the content of fatty acids in the diet and fatty acyl tail composition of membrane phospholipids. This relationship was paralleled by alterations in glucagon-stimulated adenylate cyclase activity.

Materials and methods

Animals and diets

Male Sprague–Dawley rats (Charles River Breeding Laboratories, Ottawa, Ontario, Canada) were fed purified diets for 24 days. Diets and water were supplied *ad libitum*. Rats were housed individually in a temperature- (21°C) and light-controlled room providing 12h-light/12h-dark periods. In experiment 1 the effect of different levels of fat in the diet was examined by feeding diets containing 9% (w/w) or 20% (w/w) low-erucic acid rape-seed oil. The effect of dietary fatty acid composition was examined by comparing the former diets with animals fed diets containing 9% (w/w) soya-bean oil. The basic experimental diet providing 20% (w/w) fat (Innis & Clandinin, 1980) was adjusted to equalize nutrient to energy density to maintain similar essential-nutrient densities between high- and low-fat diets. Feeding of three replicates comprising duplicate groups of two animals for each experimental diet was initiated sequentially over a 3 day period. Therefore, initial body weights for each day were 59.0 ± 2.6g, 69.8 ± 1.8g and 76.3 ± 2.6g respectively. Fatty acid composition of the oils used in experiment 1 are similar to those described previously (Clandinin, 1978; Innis & Clandinin, 1980).

Preparation of liver plasma membranes

Plasma membranes were isolated (Ray, 1970) with modification to scale-up plasma-membrane yield. After decapitation, two livers were rapidly excised, pooled and washed with 1mM-$NaHCO_3$ buffer containing 0.5mM-$CaCl_2$, pH 7.5, at 0.5°C; 6g of liver per group was cut into 2mm^3 pieces and homogenized with an electric-powered homogenizer. The homogenate was diluted 70-fold with respect to the wet weight of fresh liver with buffer. Each subsequent homogenate was diluted to half the previous volume. Membranes pelleted by differential centrifugation (Ray, 1970) were purified by sucrose-density-gradient ultracentrifugation in an SW 28 Rotor (Beckman Spinco, Palo Alto, CA, U.S.A.). Cellulose nitrate tubes containing the sample pellet and 12ml of 65% (w/w) sucrose were mixed by using a vortex mixer. The sucrose gradient was prepared by overlaying with 10ml of 44% (w/w) sucrose, 8ml of 40% (w/w) sucrose and 4ml of 37% (w/w) sucrose. Gradients were centrifuged (Beckman Spinco model L8-55 ultracentrifuge) for 2h at 25000rev./min (82700 ***g*** at $r_{av.}$) by using slow acceleration and decelerating without braking. Isolated plasma membrane was removed from the 37%/40% (w/w) sucrose interface.

In experiment 2, the dietary fat fed was prepared by combining high-linoleic acid safflower oil with different proportions of high-oleic acid safflower oil to provide a dietary fat that differed primarily in the level of linoleic acid and oleic acid present (Table 1). Five different mixtures were fed. Linseed oil (2.01%, w/w) was added to provide a dietary level of ($\omega-3$)-unsaturated fatty acid similar to that fed in experiment 1. The total fat level in the diets fed in experiment 2 was 20% (w/w). For comparative purposes the low-erucic acid diet fed in experiment 1 was also utilized in experiment 2. For each diet, three replicates comprising one rat per replicate were fed. Each replicate was initiated on a different day and the initial body weights for each replicate were 58.3 ± 0.8, 66.7 ± 2.4 and 69.5 ± 5.4g respectively.

Enzyme assay

Purity of the plasma-membrane preparation was assessed by 5′-nucleotidase, glucose 6-phosphatase

Table 1. *Effect of diet on the fatty acyl composition of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol and sphingomyelin*

Values are means ± S.D. for: 0 days, rats killed before dietary treatment; LER, rats fed 9% (w/w) or 20% (w/w) low-erucic acid rape-seed oil; SBO, rats fed 9% (w/w) soya-bean oil. Abbreviations used: Σsats, sum of saturated fatty acids; Σ(ω−9), sum of (ω−9)-unsaturated fatty acids; (ω−6), sum of (ω−6)-unsaturated fatty acids; Σ(ω−3), sum of (ω−3)-unsaturated fatty acids; Σ(ω−6)/(ω−3), ratio of total (ω−6) to total (ω−3)-unsaturated fatty acids; U.I., unsaturation index. Animal weight gain was, after 24 days of dietary treatment of 9% (w/w) LER, 20% (w/w) LER or 9% (w/w) SBO, 160.3 ± 8.89^{a} g and 175.5 ± 10.21^{b} g and 169.8 ± 5.78^{ab} g respectively. Values without a common superscript are significantly different ($P < 0.05$). Sums for the day 0 values were not statistically compared with the treatment groups (NC).

	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2(9,12)}$	$C_{18:3(9,12,15)}$	$C_{20:0}$	$C_{20:1}$	$C_{20:2(11,14)}$	$C_{20:3(8,11,14)}$	$C_{20:4(5,8,11,14)}$	$C_{20:5(5,8,11,14,17)}$	$C_{22:0}$
Phosphatidylcholine													
Day 0	26.7 ± 0.8^{a}	0.3 ± 0.1	19.3 ± 1.2^{a}	10.2 ± 1.7^{b}	13.6 ± 0.8^{a}	0.0 ± 0.1^{a}	0.0 ± 0.0	0.1 ± 0.1^{ac}	0.0 ± 0.0	0.8 ± 0.1^{a}	17.2 ± 1.7^{a}	0.0 ± 0.0	0.0 ± 0.0
9% (w/w)LER	21.7 ± 1.6^{b}	0.5 ± 0.2	22.5 ± 2.0^{b}	10.9 ± 1.5^{b}	10.9 ± 1.1^{b}	0.3 ± 0.1^{b}	0.1 ± 0.1^{ab}	0.7 ± 0.3^{bc}	0.5 ± 0.0	1.0 ± 0.2^{b}	19.6 ± 2.4^{ab}	0.2 ± 0.4	0.0 ± 0.0
20% (w/w)LER	18.8 ± 2.1^{b}	0.3 ± 0.2	22.6 ± 1.4^{b}	11.9 ± 1.0^{b}	10.7 ± 0.6^{b}	0.3 ± 0.0^{b}	0.2 ± 0.1^{a}	0.9 ± 0.3^{b}	0.5 ± 0.1	0.9 ± 0.1^{ab}	23.5 ± 2.5^{b}	0.1 ± 0.3	0.0 ± 0.0
9% (w/w)SBO	23.4 ± 2.4^{c}	0.5 ± 0.2	22.2 ± 1.9^{ab}	6.8 ± 0.6^{a}	14.3 ± 0.7^{a}	0.2 ± 0.0^{b}	0.0 ± 0.0	0.3 ± 0.1^{c}	0.6 ± 0.1	0.8 ± 0.2^{ab}	21.9 ± 3.3^{b}	0.0 ± 0.0	0.0 ± 0.0
Phosphatidylethanolamine													
Day 0	17.1 ± 2.6^{b}	0.2 ± 0.1^{bc}	24.0 ± 1.4^{a}	7.8 ± 1.1^{b}	7.4 ± 1.3^{a}	0.1 ± 0.1^{a}	0.0 ± 0.0^{b}	0.1 ± 0.1^{a}	0.0 ± 0.0	0.4 ± 0.1	21.6 ± 1.9	0.0 ± 0.0	0.0 ± 0.0
9% (w/w)LER	16.7 ± 2.3^{b}	0.3 ± 0.4^{a}	27.2 ± 1.5^{ba}	7.0 ± 1.5^{bc}	4.5 ± 1.0^{b}	0.4 ± 0.2^{b}	0.7 ± 0.1^{b}	0.7 ± 0.2^{b}	0.3 ± 0.0^{a}	0.5 ± 0.1	22.9 ± 2.3	0.2 ± 0.2	0.0 ± 0.0
20% (w/w)LER	13.0 ± 2.5^{a}	0.1 ± 0.0^{b}	28.0 ± 3.1^{b}	10.9 ± 0.8^{a}	5.3 ± 0.6^{b}	0.4 ± 0.1^{b}	0.2 ± 0.0^{a}	1.0 ± 0.1^{c}	0.4 ± 0.0^{ab}	0.5 ± 0.0	24.4 ± 4.4	0.0 ± 0.1	0.0 ± 0.0
9% (w/w)SBO	16.4 ± 1.3^{ab}	0.3 ± 0.1^{ac}	26.8 ± 2.7^{ab}	5.2 ± 0.6^{c}	7.4 ± 0.8^{a}	0.3 ± 0.1^{ab}	0.0 ± 0.0^{b}	0.4 ± 0.1^{c}	0.5 ± 0.2^{ab}	0.5 ± 0.1	25.9 ± 2.2	0.0 ± 0.0	0.0 ± 0.0
Sphingomyelin													
Day 0	7.1 ± 0.8^{a}	0.0 ± 0.0	10.4 ± 2.8	2.0 ± 0.2^{a}	1.2 ± 0.7	0.2 ± 0.3	2.2 ± 0.5	0.0 ± 0.1^{a}	0.0 ± 0.0	0.4 ± 0.5	2.6 ± 0.5^{b}	0.0 ± 0.0	10.1 ± 0.7^{ac}
9% (w/w)LER	14.1 ± 2.3^{b}	0.0 ± 0.0	8.8 ± 2.5	3.8 ± 1.4^{ab}	2.3 ± 1.0	0.5 ± 0.1	1.3 ± 0.3	0.6 ± 0.1^{b}	0.2 ± 0.2	0.3 ± 0.3	3.2 ± 0.5^{b}	0.0 ± 0.0	8.3 ± 0.9^{b}
20% (w/w)LER	13.0 ± 0.6^{b}	0.0 ± 0.0	12.1 ± 3.4	4.9 ± 0.9^{b}	3.3 ± 1.6	0.5 ± 0.1	2.2 ± 1.1	0.7 ± 0.2^{b}	0.1 ± 0.1	0.1 ± 0.1	5.3 ± 0.4^{a}	0.0 ± 0.0	8.5 ± 0.7^{ab}
9% (w/w)SBO	12.7 ± 1.6^{b}	0.0 ± 0.0	8.4 ± 1.6	2.0 ± 0.3^{a}	1.6 ± 0.2	0.6 ± 0.1	1.2 ± 0.3	0.7 ± 0.3^{b}	0.2 ± 0.1	0.0 ± 0.0	3.0 ± 0.6^{b}	0.0 ± 0.0	10.9 ± 0.5^{c}
Phosphatidylserine													
Day 0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0						
9% (w/w)LER	5.4 ± 0.9^{a}	0.0 ± 0.0	44.1 ± 5.5	7.9 ± 0.9^{a}	2.6 ± 0.6	0.6 ± 0.4	0.4 ± 0.4	1.0 ± 0.3	0.4 ± 0.2	1.0 ± 0.4	24.1 ± 5.3	0.0 ± 0.0	0.0 ± 0.0
20% (w/w)LER	3.8 ± 0.4^{b}	0.0 ± 0.0	47.6 ± 4.2	8.0 ± 0.7^{a}	1.5 ± 1.2	0.7 ± 0.2	0.2 ± 0.1	1.2 ± 0.1	0.4 ± 0.1	1.1 ± 0.2	22.9 ± 4.9	0.0 ± 0.0	0.0 ± 0.0
9% (w/w)SBO	4.6 ± 0.7^{ab}	0.0 ± 0.0	44.4 ± 5.8	5.1 ± 2.0^{b}	2.5 ± 0.4	1.0 ± 0.4	0.1 ± 0.1	1.2 ± 0.5	0.3 ± 0.2	0.9 ± 0.5	28.2 ± 4.8	0.0 ± 0.0	0.0 ± 0.0
Phosphatidylinositol													
Day 0	9.0 ± 0.6^{a}	0.0 ± 0.0	41.1 ± 3.7	4.3 ± 1.1^{b}	2.5 ± 1.4	0.0 ± 0.0^{a}	0.0 ± 0.1	0.0 ± 0.0^{a}	0.6 ± 0.1^{ab}	0.8 ± 0.1^{b}	24.4 ± 2.1	0.0 ± 0.0	0.0 ± 0.0
9% (w/w)LER	3.8 ± 0.6^{b}	0.0 ± 0.0	43.0 ± 3.9	5.4 ± 0.6^{b}	1.8 ± 0.4	0.8 ± 0.4^{b}	0.1 ± 0.2	1.0 ± 0.4^{b}	1.1 ± 0.5^{a}	1.6 ± 0.7^{a}	27.9 ± 2.4	0.0 ± 0.0	0.0 ± 0.0
20% (w/w)LER	3.8 ± 0.3^{bc}	0.0 ± 0.0	41.2 ± 3.6	8.2 ± 0.5^{a}	2.7 ± 0.5	1.0 ± 0.2^{b}	0.2 ± 0.0	1.2 ± 0.4^{b}	0.6 ± 0.1^{ab}	1.2 ± 0.1^{b}	25.9 ± 3.9	0.0 ± 0.0	0.0 ± 0.0
9% (w/w)SBO	6.4 ± 2.0^{d}	0.0 ± 0.0	40.5 ± 0.4	5.3 ± 0.9^{b}	3.1 ± 0.6	1.0 ± 0.3^{b}	0.1 ± 0.1	0.9 ± 0.2^{b}	0.4 ± 0.1^{b}	0.8 ± 0.3^{b}	29.3 ± 4.9	0.0 ± 0.0	0.0 ± 0.0

	$C_{22:1}$	$C_{22:4(7,10,13,16)}$	$C_{22:5(4,7,10,13,16,19)}$	$C_{22:5(4,7,10,13,16,19)}$	$C_{22:6}$	$C_{24:0}$	$C_{24:1}$	Σsats	Σ(ω−9)	Σ(ω−6)	Σ(ω−3)	Σ(ω−6)/(ω−3)	U.I.
Phosphatidylcholine													
Day 0	0.0 ± 0.0	0.0 ± 0.0^{a}	0.1 ± 0.1^{ab}	0.6 ± 0.1	7.1 ± 0.7^{a}	0.0 ± 0.0	0.0 ± 0.0	NC	NC	NC	NC	NC	NC
9% (w/w)LER	0.0 ± 0.0	0.0 ± 0.0^{a}	0.1 ± 0.1^{a}	0.5 ± 0.1	5.7 ± 1.2^{ab}	0.0 ± 0.0	0.0 ± 0.0	44.4 ± 2.8	12.2 ± 1.7^{a}	32.9 ± 2.1^{a}	6.7 ± 1.2	5.0 ± 0.9^{a}	159.3 ± 10.9
20% (w/w)LER	0.0 ± 0.0	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.5 ± 0.1	5.2 ± 0.9^{b}	0.0 ± 0.0	0.0 ± 0.0	41.8 ± 2.0	13.0 ± 1.7^{a}	35.9 ± 1.8^{ab}	6.1 ± 1.2	6.0 ± 1.1^{a}	168.6 ± 7.7
9% (w/w)SBO	0.0 ± 0.0	0.1 ± 0.1^{b}	0.1 ± 0.1^{b}	0.5 ± 0.1	4.2 ± 0.8^{b}	0.0 ± 0.0	0.0 ± 0.0	45.8 ± 3.5	7.7 ± 0.8^{b}	38.0 ± 3.7^{b}	5.0 ± 0.9	7.8 ± 1.1^{b}	158.8 ± 18.4
Phosphatidylethanolamine													
Day 0	0.0 ± 0.0	0.3 ± 0.1^{b}	0.3 ± 0.1^{a}	1.6 ± 0.2	14.9 ± 1.3^{a}	0.0 ± 0.0	0.0 ± 0.0	NC	NC	NC	NC	NC	NC
9% (w/w)LER	0.2 ± 0.2	0.4 ± 0.1^{b}	0.2 ± 0.1^{ab}	1.3 ± 0.7	11.7 ± 2.5^{ab}	0.0 ± 0.0	0.0 ± 0.0	44.1 ± 3.5	8.2 ± 1.6^{a}	29.2 ± 2.2^{a}	13.5 ± 2.7	2.2 ± 0.4^{a}	194.1 ± 25.0^{bc}
20% (w/w)LER	0.2 ± 0.4	0.3 ± 0.1^{b}	0.0 ± 0.0^{b}	1.3 ± 0.4	8.7 ± 2.2^{b}	0.0 ± 0.0	0.0 ± 0.0	41.2 ± 5.3	12.3 ± 0.9^{b}	31.2 ± 3.7^{ab}	10.4 ± 2.6	3.1 ± 0.4^{b}	185.6 ± 34.2^{ab}
9% (w/w)SBO	0.1 ± 0.1	0.9 ± 0.1^{a}	0.4 ± 0.2^{a}	1.7 ± 0.5	9.0 ± 1.4^{b}	0.0 ± 0.0	0.0 ± 0.0	43.3 ± 3.7	6.1 ± 0.6^{c}	35.5 ± 2.7^{b}	11.0 ± 1.7	3.3 ± 0.4^{b}	195.6 ± 18.6^{ab}
Sphingomyelin													
Day 0	0.9 ± 0.0^{ab}	10.5 ± 0.8^{a}	0.0 ± 0.0	0.3 ± 0.1^{a}	0.0 ± 0.0	23.7 ± 2.3^{a}	18.5 ± 1.5^{ab}	NC	NC	NC	NC	NC	NC
9% (w/w)LER	0.9 ± 0.1^{ab}	4.3 ± 0.5^{b}	0.3 ± 0.4	0.0 ± 0.0^{b}	0.0 ± 0.0	18.6 ± 2.4^{ab}	25.5 ± 2.9^{a}	51.2 ± 1.6^{a}	30.8 ± 2.2^{b}	10.6 ± 0.9^{a}	0.5 ± 0.1	22.8 ± 4.4	69.8 ± 2.0^{a}
20% (w/w)LER	0.6 ± 0.2^{a}	3.8 ± 0.5^{b}	0.4 ± 0.3	0.1 ± 0.1^{b}	0.0 ± 0.0	15.8 ± 2.8^{b}	23.1 ± 4.2^{a}	51.6 ± 1.0^{a}	29.3 ± 3.8^{b}	13.0 ± 1.2^{b}	0.6 ± 0.1	12.5 ± 5.0	76.5 ± 2.7^{b}
9% (w/w)SBO	1.2 ± 0.1^{b}	7.5 ± 0.2^{c}	0.5 ± 0.2	0.0 ± 0.0^{b}	0.0 ± 0.0	25.5 ± 1.1^{ac}	9.2 ± 6.7^{b}	58.8 ± 1.9^{b}	13.2 ± 6.7^{a}	12.9 ± 0.5^{b}	0.6 ± 0.1	23.6 ± 5.7	63.4 ± 5.9^{c}
Phosphatidylserine													
Day 0		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	NC	NC	NC	NC	NC	NC
9% (w/w)LER	0.4 ± 0.3	0.1 ± 0.2	0.0 ± 0.0	0.6 ± 0.3	5.0 ± 1.1^{a}	0.4 ± 0.6	0.3 ± 0.1	50.3 ± 5.3	9.7 ± 1.5^{ab}	28.3 ± 6.1^{ab}	6.2 ± 1.0^{a}	4.5 ± 0.9^{a}	150.6 ± 28.2
20% (w/w)LER	0.6 ± 0.1	0.2 ± 0.1	0.0 ± 0.0	0.8 ± 0.3	3.0 ± 0.9^{b}	0.2 ± 0.2	0.4 ± 0.1	51.8 ± 4.5	12.1 ± 0.9^{a}	76.0 ± 5.4^{a}	4.5 ± 0.8^{b}	5.8 ± 0.7^{b}	135.5 ± 24.8
9% (w/w)SBO	0.7 ± 0.3	0.4 ± 0.1	0.0 ± 0.0	0.6 ± 0.1	3.3 ± 0.9^{ab}	0.2 ± 0.3	0.4 ± 0.2	49.3 ± 6.1	7.5 ± 2.5^{b}	32.5 ± 5.4^{b}	4.9 ± 0.7^{b}	6.6 ± 0.6^{c}	156.3 ± 24.3
Phosphatidylinositol													
Day 0	0.0 ± 0.0^{a}	0.5 ± 0.2	0.4 ± 0.1^{a}	1.7 ± 1.1	11.4 ± 1.4^{a}	0.0 ± 0.0	0.0 ± 0.0^{a}	NC	NC	NC	NC	NC	NC
9% (w/w)LER	0.5 ± 0.4^{b}	0.4 ± 0.1	0.2 ± 0.1^{ab}	1.0 ± 0.3	5.0 ± 1.4^{b}	0.0 ± 0.0	0.4 ± 0.2^{b}	47.0 ± 4.1	7.4 ± 0.9^{a}	33.1 ± 3.1	6.9 ± 1.2	5.6 ± 0.5	147.1 ± 13.7
20% (w/w)LER	0.7 ± 0.1^{b}	0.3 ± 0.1	0.2 ± 0.0^{b}	0.9 ± 0.2	3.7 ± 0.4^{b}	0.0 ± 0.0	0.6 ± 0.2^{b}	45.1 ± 3.4	10.6 ± 1.0^{b}	30.8 ± 3.9	4.9 ± 1.0	5.5 ± 0.3	140.6 ± 16.9
9% (w/w)SBO	0.6 ± 0.3^{b}	0.4 ± 0.1	0.3 ± 0.1^{ab}	0.8 ± 0.2	3.8 ± 0.7^{b}	0.0 ± 0.0	0.5 ± 0.2^{b}	46.1 ± 2.3	7.3 ± 1.4^{a}	34.3 ± 4.3	5.6 ± 0.6	6.2 ± 1.2	150.3 ± 17.0

(Aronson & Touster, 1974) and monoamine oxidase activities (Schnaitman *et al.*, 1967). Activity obtained for 5'-nucleotidase, glucose 6-phosphatase and monoamine oxidase was 30.9 μmol/mg of protein per h, 3.9 μmol/mg of protein per h and 0.009 unit/mg of protein respectively. These activities are consistent with previous reports (Emmelot *et al.*, 1964; Coleman *et al.*, 1967; Ray, 1970; Yunghans & Morre, 1973) and indicate low contamination of the plasma-membrane preparation by smooth endoplasmic reticulum or mitochondria. These conclusions were also confirmed by electron microscopy. Plasma-membrane yields of 0.67–1.3 mg of protein/g wet wt. of liver were obtained, which were similar to those reported previously (Ray, 1970).

Adenylate cyclase was assayed (Solomon *et al.*, 1974) with the following minor modifications; the assay mixture contained 5 mM-Tris/HCl (pH 7.5), 5 mM-$MgCl_2$, 20 mM-phosphocreatine, 100 μmol of creatine kinase/ml, 100 μM-GTP, 1 mM- or 50 nM-glucagon and ATP concentrations as indicated in Fig. 1. The reaction was initiated at 30°C by addition of 30 μg of membrane protein in a final reaction volume of 100 μl. The reaction was terminated after 7.5 min by immersion in a boiling-water bath. Cyclic AMP was assayed by a competitive binding assay kit (Amersham, Oakville, Ontario, Canada). Portions of plasma-membrane preparations for adenylate cyclase assay were frozen on solid CO_2 for experiment 1 and in liquid N_2 for experiment 2 and stored at −70°C. Protein was measured by a colorimetric method (Lowry *et al.*, 1951).

Lipid analysis

Plasma-membrane lipids were extracted by a modified Folch extraction procedure. Phospholipids were purified from neutral lipids by t.l.c. on silica-gel G plates and individual phospholipids were separated by t.l.c. on silica-gel H plates (Innis & Clandinin, 1981*a*) with the following modifications to handle the large number of samples and to improve phospholipid separation. A single solvent system of light petroleum (b.p. 72.4–107°C)/diethyl ether/ acetic acid (85:15:1, by vol.) was used for phospholipid purification. Chloroform/acetone/methanol/acetic acid/water (30:40:10:10:3, by vol.) was used in the second solvent system in two-dimensional separation of phospholipids. Phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol and sphingomyelin were identified and recovered. Phospholipid fatty acids were converted into methyl esters by using BF_3/methanol reagent (Morrison & Smith, 1961). Fatty acid methyl esters were separated by automated g.l.c. utilizing an open tubular glass capillary column (18 m × 0.25 mm internal diameter) as described previously (Innis & Clandinin, 1981*a*).

Statistical analysis

The effect of dietary treatment in experiment 1 was examined by multivariate-analysis-of-variance techniques (Steel & Torrie, 1960). Comparison between individual diets was made by using Neuman–Keuls multiple-range test after an effect of diet treatment was established by analysis of variance. For experiment 2 relationships were examined by regression analysis.

Results and discussion

The fatty acyl composition of phosphatidylcholine, phosphatidylethanolamine, sphingomyelin, phosphatidylinositol and phosphatidylserine differ greatly from each other (Table 1) (Keenan & Morre, 1970; Van Hoeven *et al.*, 1975; Holub & Kuksis, 1978). Results of this study show that phosphatidylethanolamine contained larger amounts of $C_{20:4}$ than $C_{18:2}$, $C_{16:0}$, $C_{18:0}$ or $C_{22:6}$ fatty acids. Phosphatidylcholine contained large amounts of $C_{18:2}$, $C_{20:4}$, $C_{16:0}$, $C_{18:0}$ and $C_{18:1}$ fatty acids. Sphingomyelin contained a much higher amount of $C_{18:0}$, $C_{22:0}$, $C_{24:0}$ and $C_{24:1}$ fatty acids than other phospholipids. Phosphatidylinositol and phosphatidylserine were rich in $C_{18:0}$ and $C_{20:4}$ fatty acids, with these two making up approx. 70% of total fatty acids.

Developmental effects

The fatty acyl composition of phosphatidylcholine, phosphatidylethanolamine, sphingomyelin and phosphatidylinositol was determined at day 0 (weaning) and was compared with the composition at day 24 to determine the effect of aging and change in diet at weaning on plasma-membrane fatty acyl composition (Table 1). Membranes from rats at day 0 contained significantly greater $C_{14:0}$ and $C_{16:0}$ and less $C_{18:3(9,12,15)}$ in phosphatidylcholine than those from 'day-24 rats', irrespective of the diet fed. For phosphatidylethanolamine, membranes from 'day-0 rats' contained less $C_{20:1}$ fatty acid than those from day-24 rats. For day-0 rats, sphingomyelin fatty acyl composition differed from day-24 rats in that the day-0 rat membranes contained lower levels of $C_{16:0}$ and $C_{20:1}$ and greater amounts of $C_{22:4(7,10,13,16)}$, $C_{22:5(7,10,13,16,19)}$ and total ($\omega-6$)-unsaturated fatty acids. At day 0, phosphatidylinositol fatty acyl composition differed in several respects from day 24. Greater amounts of $C_{16:1}$, $C_{22:6(4,7,10,13,16,19)}$ and total ($\omega-3$)-unsaturated fatty acids and lower amounts of $C_{18:3(9,12,15)}$, $C_{20:1}$ and $C_{22:1}$ fatty acids were observed. No data were

obtained for the fatty acid composition of phosphatidylserine at day 0. Differences in membrane fatty acid composition between day-0 and day-24 rats suggest that specific changes in membrane composition are due to developmental changes within the animal and the change in diet at weaning.

Dietary effects on phospholipid fatty acyl composition

Phosphatidylethanolamine. Comparison of low- and high-fat low-erucic acid rape-seed oil treatments (experiment 1) shows that the content of total ($\omega-9$)-unsaturated fatty acids and the ($\omega-6$)/($\omega-3$)-unsaturated fatty acid ratio were lower in plasma-membrane phosphatidylethanolamine for animals fed low-fat treatments (Table 1). Higher total ($\omega-9$)-unsaturated fatty acids, lower total ($\omega-6$)-unsaturated fatty acids and lower ($\omega-6$)/($\omega-3$)-unsaturated fatty acid ratios were apparent for animals fed 9% (w/w) low-erucic acid rape-seed oil when compared with those fed 9% (w/w) soya-bean oil. Both fatty acid composition of dietary fat and fat level affects the fatty acid composition of phosphatidylethanolamine. Feeding rats 20% (w/w) low-erucic acid rape-seed oil produces higher total ($\omega-9$)-unsaturated fatty acid and lower $C_{16:0}$ fatty acid content in plasma-membrane phosphatidylethanolamine (Table 1). No dietary treatment had a significant effect on total saturated and ($\omega-3$)-unsaturated fatty acid content or unsaturation index of phosphatidylethanolamine.

Phosphatidylcholine. The level of fat intake had no effect on the fatty acid composition of phosphatidylcholine. Irrespective of dietary level, feeding the low-erucic acid rape-seed oil increased the content of $C_{18:1}$ and total ($\omega-9$)-unsaturated fatty acids and lowered $C_{16:0}$ and the ratio of ($\omega-6$)/($\omega-3$)-unsaturated fatty acids compared with soya-bean oil treatments. Low-fat low-erucic acid rape-seed oil treatments also lowered the ($\omega-6$)-unsaturated fatty acid content compared with dietary soya-bean oil.

Sphingomyelin. $C_{20:4}$, total ($\omega-6$)-unsaturated fatty acids and the unsaturation index were lower in sphingomyelin for low-fat compared with high-fat treatments. Sphingomyelin isolated from plasma membranes of rats fed low-erucic acid rape-seed oil at both levels of dietary fat showed lower $C_{22:4}$ and saturated fatty acid content, higher levels of total ($\omega-9$)-unsaturated fatty acids, particularly $C_{24:1}$, and a higher unsaturation index than that observed for rats fed soya-bean oil.

Phosphatidylinositol. Higher dietary fat intake increased total ($\omega-9$)-unsaturated fatty acid content, particularly $C_{18:1}$ fatty acid, relative to the low-fat diet, irrespective of fatty acid composition of the low-fat diet fed. No effect of diet on saturated and ($\omega-6$) and ($\omega-3$) fatty acid content or unsaturation index was observed for phosphatidylinositol.

Phosphatidylserine. Unlike other phospholipids examined the level of total ($\omega-3$)-unsaturated fatty acids in phosphatidylserine was altered by diet. Total ($\omega-3$)-unsaturated fatty acid levels were higher for phosphatidylserine from rats fed 9% (w/w) low-erucic acid rape-seed oil compared with those fed either 20% (w/w) low-erucic acid rape-seed oil or 9% (w/w) soya-bean oil. The ratio of ($\omega-6$)/($\omega-3$)-unsaturated fatty acids was significantly different for each diet treatment, with 9% (w/w) low-erucic acid rape-seed oil resulting in the lowest ratio and 9% (w/w) soya-bean oil resulting in the highest ratio.

Plasma membrane phospholipid fatty acyl composition appears to reflect, in part, dietary fatty acid composition (Table 1). Phosphatidylethanolamine, phosphatidylcholine and sphingomyelin from rats fed low-erucic acid rape-seed oil have greater levels of ($\omega-9$) and lower levels of ($\omega-6$)-unsaturated fatty acids than observed for animals fed soya-bean oil. Other treatment differences in phospholipid fatty acid composition appear to be associated with a specific phospholipid (for example, changes in ($\omega-3$)-unsaturated fatty acid content of phosphatidylserine, unsaturation index of sphingomyelin).

Dietary fat level affects phospholipid fatty acid composition and the reason for this affect is not apparent. Lipogenesis is decreased in animals fed a high-fat diet. Thus fatty acids incorporated into membranes would originate to a greater extent from diet or from stored fatty acids. In animals fed low-fat diets more of the fatty acid incorporated into membranes might be expected to originate from synthesis *de novo*. For example, in phosphatidylethanolamine, $C_{18:1}$ and total ($\omega-9$)-unsaturated fatty acid level increased and $C_{16:0}$ fatty acid decreased on the high-fat diet compared with the low-fat low-erucic acid diet. Thus the fatty acid composition of phosphatidylethanolamine for the high-fat treatment reflected the fatty acid composition of the diet to a greater degree than for the lower-fat diet. It is apparent that the fatty acid composition of plasma-membrane lipid composition is under intrinsic control but is also sensitive to extrinsic influences such as diet.

Diet effects on glucagon-stimulated adenylate cyclase activity

Dietary treatment resulted in significant differences in glucagon-stimulated adenylate cyclase activity at glucagon concentrations of 1 mM where near maximal responses can be obtained (Fig. 1). The high-fat diet resulted in lower glucagon-stimulated adenylate cyclase activity at 5 mM- and 7.5 mM-ATP compared with that for animals fed 9%

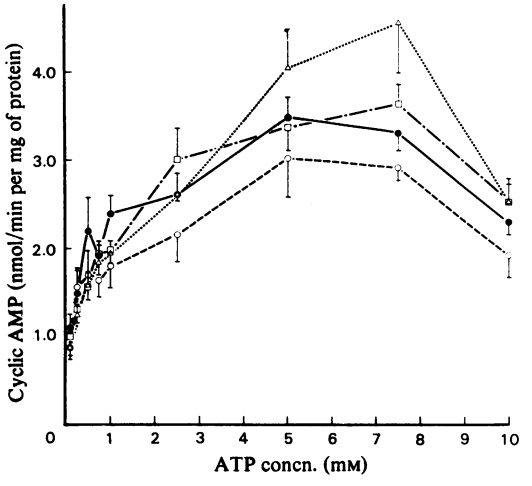

Fig. 1. *Effect of ATP concentration on cyclic AMP synthesis by glucagon-stimulated adenylate cyclase from rats fed high- and low-fat treatments*

Adenylate cyclase activity was assayed in triplicate in the presence of 1 mM-glucagon as described in the text for six groups of rats from each diet treatment. Isolated plasma membranes were assayed from animals at weaning (day 0; ●), and after 24 days of feeding diet containing either 9% (w/w) low-erucic acid rape-seed oil (△), 20% (w/w) low-erucic acid rape-seed oil (○) or 9% (w/w) soya-bean oil (□). The effect of diet fat is statistically significant ($P < 0.05$).

(w/w) soya-bean oil or low-erucic acid rape-seed oil. Glucagon-stimulated adenylate cyclase activity for animals at weaning was significantly lower at 7.5 mM-ATP than the activity observed for rats fed 9% (w/w) low-erucic acid rape-seed oil for 24 days. To examine whether these differences in activity also existed at low concentrations of glucagon, the assay was repeated for previously frozen samples of membrane using concentrations of 1 mM- and 50 nM-glucagon (Table 2). At these glucagon concentrations glucagon-stimulated adenylate cyclase activity was greater for animals fed the low-fat low-erucic acid rape-seed oil diet. However, at low glucagon concentrations glucagon-stimulated adenylate cyclase activity for rats fed 9% (w/w) low-erucic acid rape-seed oil was not significantly different from that observed for animals fed 9% (w/w) soya-bean oil. Glucagon-stimulated adenylate cyclase activities for rats fed 9% (w/w) and 20% (w/w) low-erucic acid rape-seed oil are thus significantly different within physiological ranges of circulating glucagon concentration (Pezzino *et al.*, 1981).

A second experiment was designed to determine if a dose–response relationship exists between the level

Table 2. *Effect of diet on glucagon-stimulated adenylate cyclase activity of rat liver plasma membrane from animals fed low-erucic acid rape-seed oil or soya-bean oil*

Results are means ± S.D. of groups assayed in Fig. 1. and are expressed as percentages of the velocity observed for 9% (w/w) low-erucic acid rape-seed oil treatment at 1 mM-glucagon concentration and 7.5 mM-ATP (4720 ± 1140 pmol of cyclic AMP synthesized/mg of protein per 7.5 min). Values at each glucagon concentration without a common superscript are significantly different ($P < 0.05$). Experimental details are given in the text.

	Glucagon-stimulated adenylate cyclase activity (%)	
Dietary treatment	1 mM-Glucagon	50 nM-Glucagon
9% (w/w) low-erucic acid rape-seed oil	72 ± 18[a]	44 ± 17[a]
20% (w/w) low-erucic acid rape-seed oil	42 ± 21[b]	36 ± 15[b]
9% (w/w) soya-bean oil	84 ± 21[a]	56 ± 20[a]

Table 3. *Fatty acid composition of dietary fat fed in experiment 2*

Diets contained 2.06% (w/w) linseed oil and a total of 17.9% (w/w) safflower oil. High-linoleic and high-oleic acid safflower oils were combined to make dietary fats of differing fatty acid composition. Fatty acid composition of oils fed is expressed as g of fatty acid per 4184 kJ (1000 kcal) of diet and was determined by laboratory analysis of the diet fat fed.

	Fatty acid composition (g/4184 kJ of diet)				
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
$C_{16:0}$	2.7	2.5	2.4	2.3	2.2
$C_{18:0}$	1.0	0.9	0.9	0.9	0.9
$C_{18:1}$	4.9	9.5	14.4	19.5	24.8
$C_{18:2}$	27.3	22.9	17.9	12.9	7.6
$C_{18:3}$	2.3	2.3	2.4	2.5	2.5

of fatty acids in the diet and membrane phospholipid fatty acyl tail composition and to examine if these changes in membrane polar lipid composition are associated with diet-induced changes in glucagon-stimulated adenylate cyclase activity. To facilitate these comparisons the fatty acid composition of the diets fed were expressed as g of fatty acid per unit of energy in the diet (Table 3).

Relationship between dietary intake and phospholipid fatty acid composition

The fatty acyl composition of phosphatidylcholine, phosphatidylethanolamine, sphingomyelin, phosphatidylserine and phosphatidylinositol was determined for liver plasma membranes of rats fed

Table 4. *Fatty acid composition of phosphatidylcholine, phosphatidylethanolamine, sphingomyelin, phosphatidylserine and phosphatidylinositol isolated from liver plasma membrane of rats fed diets in experiment 2*

Values are grand means ± S.D. for all diet treatments. Abbreviation used: Σmonosats, sum of monounsaturated fatty acids. Other abbreviations are defined in the legend to Table 1. The significance level for an effect of diet treatment is indicated as follows: *, $P<0.05$; **, $P<0.01$; ***, $P<0.0001$, and was determined by analysis-of-variance procedures.

Fatty acid	Phosphatidyl-choline	Phosphatidyl-ethanolamine	Sphingomyelin	Phosphatidyl-serine	Phosphatidyl-inositol
$C_{16:0}$	21.98 ± 1.57	14.22 ± 0.48	15.99 ± 1.62	5.00 ± 1.13	9.04 ± 0.45
$C_{16:1}$	0.22 ± 0.06	0.13 ± 0.06	0.06 ± 0.13	0.0 ± 0.0	0.71 ± 0.88
$C_{18:0}$	23.04 ± 0.91	27.94 ± 1.10	12.48 ± 2.40	58.12 ± 6.41	45.50 ± 5.39
$C_{18:1}$	7.63 ± 3.45*	6.77 ± 1.57*	2.10 ± 0.64	5.16 ± 1.99	6.88 ± 2.36
$C_{18:2(9,12)}$	9.89 ± 1.32*	6.06 ± 0.91	1.71 ± 0.93	2.24 ± 0.69	2.16 ± 0.32
$C_{18:3(9,12,15)}$	0.11 ± 0.04	0.29 ± 0.10	0.43 ± 0.50*	1.19 ± 0.70	3.94 ± 1.47
$C_{20:0}$	0.18 ± 0.02	0.22 ± 0.07	1.44 ± 0.39	0.16 ± 0.16	0.0 ± 0.0
$C_{20:1}$	0.46 ± 0.23*	0.54 ± 0.18	0.22 ± 0.27	0.0 ± 0.0	0.0 ± 0.0
$C_{20:2(11,14)}$	1.08 ± 0.50***	0.98 ± 0.45*	0.35 ± 0.34*	0.08 ± 0.15	0.0 ± 0.0
$C_{20:3(8,11,14)}$	0.61 ± 0.10**	0.40 ± 0.06	0.15 ± 0.15*	0.53 ± 0.25	0.0 ± 0.0
$C_{20:4(5,8,11,14)}$	25.10 ± 3.18	24.88 ± 1.15	3.95 ± 1.02	20.63 ± 6.32	18.07 ± 2.93
$C_{20:4(8,11,14,17)}$	0.10 ± 0.11*	0.17 ± 0.16*	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
$C_{20:5(5,8,11,14,17)}$	0.02 ± 0.02	0.02 ± 0.02	0.11 ± 0.11	0.02 ± 0.05	0.0 ± 0.0
$C_{22:0}$	0.0 ± 0.0	0.0 ± 0.0	10.10 ± 0.99	0.0 ± 0.0	0.0 ± 0.0
$C_{22:4(7,10,13,16)}$	0.25 ± 0.15	1.01 ± 0.29	5.46 ± 0.73	0.29 ± 0.30*	0.0 ± 0.0
$C_{22:5(4,7,10,13,16)}$	0.36 ± 0.29	0.47 ± 0.26	0.10 ± 0.08	0.0 ± 0.0	0.0 ± 0.0
$C_{22:5(7,10,13,16,19)}$	0.41 ± 0.15	0.87 ± 0.26	0.0 ± 0.0	0.07 ± 0.13	0.0 ± 0.0
$C_{22:6(4,7,10,13,16,19)}$	5.29 ± 1.11	10.53 ± 1.23	0.0 ± 0.0	2.59 ± 0.93	3.69 ± 2.07
$C_{24:0}$	0.0 ± 0.0	0.0 ± 0.0	24.75 ± 2.26	0.0 ± 0.0	0.0 ± 0.0
$C_{24:1}$	0.17 ± 0.09	0.58 ± 0.50	15.37 ± 3.17	1.67 ± 0.98	3.00 ± 0.69
Σsats	45.20 ± 1.76	42.38 ± 1.20	64.35 ± 2.72	63.28 ± 6.92	54.55 ± 5.15
Σmonosats	8.48 ± 3.72*	8.03 ± 1.61	17.74 ± 3.16	6.83 ± 2.85	10.59 ± 3.08
$\Sigma(\omega-6)$	37.28 ± 4.18*	33.80 ± 1.41	11.74 ± 1.77	23.77 ± 7.23	20.23 ± 2.93
$\Sigma(\omega-3)$	5.93 ± 1.18	11.87 ± 1.36	0.54 ± 0.47	3.88 ± 0.89	7.63 ± 1.13
U.I.	170.03 ± 16.10	198.34 ± 7.82	62.33 ± 3.68	116.36 ± 29.50	121.11 ± 16.90

for 24 days in experiment 2 and are expressed as grand means for all diet treatments (Table 4). The fatty acyl composition of phosphatidylcholine, phosphatidylethanolamine, sphingomyelin and phosphatidylserine was regressed compared with the dietary level of oleic acid. Significant linear regression equations ($P<0.05$) are presented (Table 5), indicating that dietary oleic acid influences the level of $C_{18:1}$, $C_{18:2(9,12)}$, $C_{20:1}$, $C_{20:3(8,11,14)}$, $C_{20:4(5,8,11,14)}$, $C_{20:4(8,11,14,17)}$ and $C_{22:5(4,7,10,13,16)}$ fatty acids in phosphatidylcholine. In phosphatidylethanolamine, dietary oleic acid influences the level of $C_{18:1}$, $C_{18:2(9,12)}$, $C_{20:2(11,14)}$, $C_{20:4(8,11,14,17)}$, $C_{22:4(7,10,13,16)}$, $C_{22:5(4,7,10,13,16)}$ and $C_{22:6(4,7,10,13,16,19)}$ fatty acids. Linear regression equations also indicate that dietary oleic acid influences the level of $C_{20:2(11,14)}$, $C_{20:3(8,11,14)}$ and $C_{24:1}$ fatty acids in sphingomyelin and the level of $C_{18:1}$, $C_{20:2(11,14)}$ and $C_{22:4(8,10,13,16)}$ fatty acids in phosphatidylserine. As observed in experiment 1, the effect of dietary oleic acid on membrane phospholipid fatty acyl composition varies considerably with the phospholipid examined. Increasing levels of this fatty acid in the diet appear to be paralleled by increasing levels of this fatty acid in membrane phospholipids, particularly for phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine. This effect was also observed in looking at the sum of the monounsaturated fatty acids in membrane phosphatidylcholine, phosphatidylethanolamine, sphingomyelin and phosphatidylserine. The level of monounsaturated fatty acids increases with increasing amounts of dietary oleic acid. As the level of oleic acid increases across the diets, there is a corresponding decrease in the dietary level of linoleic acid. This aspect of fatty acid balance is also reflected in membrane phospholipid fatty acyl composition. The level of linoleic acid in membrane phosphatidylcholine and phosphatidylethanolamine decreases as the level of oleic acid in the diet increases. A similar effect is also seen with the sum of the $(\omega-6)$-unsaturated fatty acid. Representative linear regression lines were constructed for fatty acid content of phosphatidylcholine, phosphatidylethanolamine or sphingomyelin using the dietary levels of $C_{18:1}$ fatty acid expressed as g per 4184 kJ of diet and are illustrated

Table 5. *Regression equations representing the influence of dietary fatty acid balance fed on phospholipid fatty acyl tail composition*

Values are given for linear regression between fatty acyl content (% of fatty acids) of phosphatidylcholine, phosphatidylethanolamine, sphingomyelin or phosphatidylserine and g of oleic acid in diet (x). Values in parentheses indicate standard deviations. Degrees of freedom for linear regression equations for phosphatidylcholine, phosphatidylethanolamine, sphingomyelin and phosphatidylserine are 9, 6, 6 and 6 respectively. Abbreviation used: nr, linear regression equation could not be fitted to the data points ($P<0.05$). Other abbreviations are defined in the legends to Tables 1 and 4. Linear regression equation could not be fitted to data points for those fatty acids listed in Table 3 but not represented in Table 4. Regression coefficients for equations given have a P value <0.05.

	Phosphatidylcholine	Phosphatidylethanolamine	Sphingomyelin	Phosphatidylserine
$C_{18:1}$	$1.68+0.407x$ (0.079)	$3.68+0.212x$ (0.029)	nr	$1.78+0.232x$ (0.072)
$C_{18:2(9,12)}$	$11.9-0.137x$ (0.040)	$7.77+0.177x$ (0.024)	nr	nr
$C_{20:1}$	$0.12+0.023x$ (0.0074)	nr	nr	nr
$C_{20:2(11,14)}$	nr	$1.77-0.0537x$ (0.016)	$0.96-0.0412x$ (0.011)	$-0.15+0.0159x$ (0.006)
$C_{20:3(8,11,14)}$	$0.48+0.009x$ (0.011)	nr	$0.40-0.0166x$ (0.006)	nr
$C_{20:4(5,8,11,14)}$	$29.1-0.272$ (0.12)	nr	nr	nr
$C_{20:4(8,11,14,17)}$	$-0.06+0.0112x$ (0.0034)	$-0.0921+0.0177x$ (0.0062)	nr	nr
$C_{22:4(7,10,13,16)}$	nr	$1.49+0.0334x$ (0.011)	nr	$0.76+0.0317x$ (0.012)
$C_{22:5(4,7,10,13,16)}$	$0.72-0.0246x$ (0.011)	$0.91-0.0306x$ (0.010)	nr	nr
$C_{22:6(4,7,10,13,16,19)}$	nr	$8.46-0.142x$ (0.045)	nr	nr
$C_{24:1}$	nr	nr	$10.2+0.354x$ (0.123)	nr
Σmonosats	$2.07+0.438x$ (0.085)	$4.92+0.214x$ (0.036)	$12.2+0.383x$ (0.103)	$2.21+0.317x$ (0.112)
$\Sigma(\omega-3)$	$44.5-0.491x$ (0.096)	$36.0-0.152x$ (0.058)	nr	nr
$\Sigma(\omega-3)$	nr	$9.29+0.177x$ (0.034)	nr	$2.47+0.0967x$ (0.037)
U.I.	nr	$185.0+0.936x$ (0.265)	nr	nr

in Fig. 2. A sixth diet, which was equivalent to the diet fed in experiment 1 [20% (w/w) low-erucic acid rape-seed oil], was given to compare observations obtained in experiment 1 with those of experiment 2. The results for this diet are also illustrated in Fig. 2.

Relationship between diet, phospholipid fatty acid composition and glucagon-stimulated adenylate cyclase activity

To examine the relationship between membrane compositional changes and enzyme activity, regression lines were constructed between plasma-membrane phosphatidylcholine, phosphatidylethanolamine and sphingomyelin fatty acid content and glucagon-stimulated adenylate cyclase activity. Significant regression equations ($P<0.05$), standard deviations and variance (%) are given (Table 6). The unsaturation index of phosphatidylethanolamine changes in concert with glucagon-stimulated adenylate cyclase activity. Increase in the unsaturation index is associated with an increase in glucagon-stimulated adenylate cyclase activity. Regression lines are illustrated for fatty acid content of phosphatidylcholine, phosphatidylethanolamine or sphingomyelin versus the glucagon-stimulated adenylate cyclase activity observed (Fig. 3). The results obtained for the sixth diet, containing 20% (w/w) low-erucic acid rape-seed oil, equivalent to the diet fed in experiment 1, are also illustrated for comparison (Fig. 3). Although the influence of membrane phospholipid fatty acyl composition on glucagon-stimulated adenylate cyclase activity varies with the fatty acid and phospholipid examined, membrane phospholipid fatty acyl com-

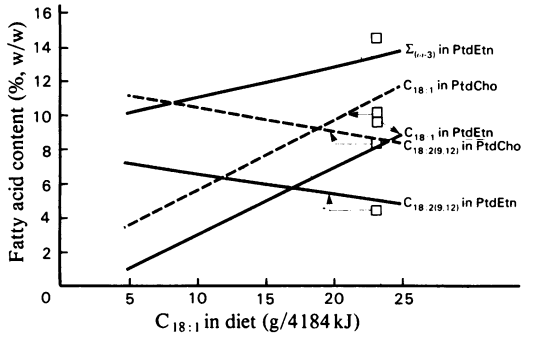

Fig. 2. *Relationship between dietary oleic acid and changes in fatty acid composition of phosphatidylcholine and phosphatidylethanolamine*

Regression lines were constructed for fatty acid content (%, w/w) of phosphatidylcholine and phosphatidylethanolamine isolated from liver plasma membrane of rats using the different dietary level of $C_{18:1}$ fatty acid fed (g of $C_{18:1}$ fatty acid per 4184 kJ of diet). Equations for these regression lines and standard deviations for each line are shown in Table 5. Another dietary treatment (low-erucic acid rape-seed oil; □) equivalent to the same diet fat fed in experiment 1 is also shown to compare observations obtained in experiment 1 with those of experiment 2. Abbreviations used: PtdEtn, phosphatidylethanolamine; PtdCho, phosphatidylcholine; $\Sigma(\omega-3)$, sum of $(\omega-3)$-unsaturated fatty acids.

Table 6. *Regression equations representing the relationship between changes in plasma-membrane phospholipid fatty acyl composition and glucagon-stimulated adenylate cyclase activity*

Values are given for linear regression between plasma-membrane phosphatidylcholine, phosphatidylethanolamine and sphingomyelin fatty acyl content (y = % of total fatty acids) and glucagon-stimulated adenylate cyclase activity (x = pmol of cyclic AMP synthesized). The standard deviation from the regression line is indicated in parentheses. Degrees of freedom for linear regression equations for phosphatidylcholine, phosphatidylethanolamine and sphingomyelin are 8, 6 and 6 respectively. 'Variance' indicates % of variance explained by linear regression equation. Abbreviations are defined in the legend to Table 1. P value is <0.05 unless indicated otherwise (* = $P < 0.01$). Linear regression equations could not be fitted to data points for fatty acids not listed.

Fatty acid	Linear regression	Variance (%)
Phosphatidylcholine		
$C_{18:1}$	$-3.19+0.0021x$ (0.00068)	47.3
$C_{20:1}$	$-0.203+0.0001x$ (0.00005)	37.0
$C_{20:2(11,14)}$	$-2.45-0.0003x$ (0.00011)	34.4
$C_{20:4(5,8,11,14)}$	$33.9-0.0017x$ (0.00070)	34.2
$\Sigma(\omega-9)$	$-3.27+0.0022x$ (0.00073)	48.2
$\Sigma(\omega-6)$	$50.4-0.0025x$ (0.00083)	47.0
Phosphatidylethanolamine		
$C_{18:1}$	$-4.70+0.0023x$ (0.00084)	52.5
$C_{18:2(9,12)}$	$-13.5-0.0015x$ (0.00038)	70.6
$C_{22:6(4,7,10,13,16,19)}$	$1.63+0.0018x$ (0.00067)	50.7
$\Sigma(\omega-9)$	$-3.48+0.0023x$ (0.00089)	49.1
$\Sigma(\omega-3)$	$2.16+0.0020x$ (0.00076)	48.9
U.I.	$133.0+0.0132x$ (0.00304)	75.0*
Sphingomyelin		
$C_{16:1}$	$0.77-0.0001x$ (0.00004)	53.2
$C_{18:1}$	$-1.11+0.0007x$ (0.00025)	64.6
$C_{20:0}$	$3.43-0.0004x$ (0.00015)	50.4
$C_{20:2(11,14)}$	$2.15-0.0004x$ (0.00012)	61.0
$C_{20:3(8,11,14)}$	$0.948-0.0002x$ (0.00006)	56.2

position does influence glucagon-stimulated adenylate cyclase activity. The level of $C_{18:1}$ and total monounsaturated fatty acids in plasma-membrane phosphatidylcholine and phosphatidylethanolamine is correlated with glucagon-stimulated adenylate cyclase activity. As the level of $C_{18:1}$ and total monounsaturated fatty acids in phosphatidylcholine and phosphatidylethanolamine increases, there is a corresponding increase in glucagon-stimulated adenylate cyclase activity. The level of total $(\omega-6)$-unsaturated fatty acids in phosphatidylcholine is inversely correlated with glucagon-stimulated adenylate cyclase. Thus, as the total $(\omega-6)$-unsaturated fatty acid level increases in the membrane, glucagon-stimulated adenylate cyclase activity decreases.

The results of experiment 2 suggest that there is a dose–response relationship between the level of oleic acid in the diet (and thus the balance of other fatty acids fed) and the membrane lipid composition. This relationship is paralleled by changes in glucagon-stimulated adenylate cyclase activity. The requirement for essential fatty acids in membranes is well established. Results of experiment 2 now indicate an effect of non-essential dietary fatty acids on membrane composition. The dietary level of these fatty acids is apparently related to changes in enzyme activity. For example, increasing the level of oleic acid in the diet results in an increase in the level of oleic acid in membrane phosphatidylcholine and this is associated with altered glucagon-stimulated adenylate cyclase activity.

It is difficult to compare these membrane changes with results obtained from studies *in vitro* altering membrane composition through growth-medium manipulations, as in the latter studies only one fatty acid was altered in the membrane (Poon *et al.*, 1981). In the whole-body feeding experiments described herein simultaneous alterations of several

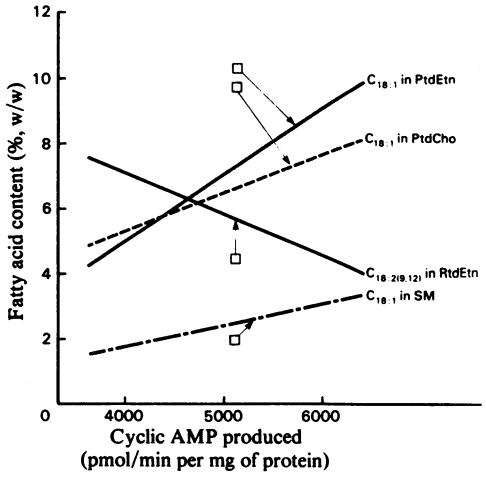

Fig. 3. *Relationship between glucagon-stimulated adenylate cyclase activity and the fatty acid composition of phosphatidylcholine (PtdCho), phosphatidylethanolamine (PtdEtn) and sphingomyelin (SM)*

Regression lines were constructed for fatty acid content (%, w/w) of phosphatidylcholine, phosphatidylethanolamine or sphingomyelin versus the glucagon-stimulated adenylate cyclase activity observed. Equations for these lines and standard deviations for data from each line are given in Table 6. A sixth diet was fed containing 20% (w/w) fat (low-erucic acid rape-seed oil; □). equivalent to the same diet fed in experiment 1, which is also illustrated to compare observations obtained in experiment 1 with those of experiment 2.

fatty acids in mode than one polar lipid occurred. Some of these observations could conceivably be directly correlated with glucagon-stimulated adenylate cyclase activity (Table 6, Fig. 3).

Rethy *et al.* (1972) and Rubakcava & Rodbell (1973) suggest that there is a role for acidic phospholipids in the glucagon-stimulated adenylate cyclase system. The results of the present study suggest that the fatty acyl tail composition of phosphatidylcholine, phosphatidylethanolamine and sphingomyelin is important in the glucagon-stimulated adenylate cyclase system.

From the present study it is apparent that the fatty acyl tail composition of liver plasma-membrane phospholipids and hormone-receptor-mediated enzyme function can be altered through manipulation of the fat component in a nutritionally complete diet. Essential-fatty-acid-deficient diets (Liepkalns & Spector, 1975; Sun *et al.*, 1979) and essential-fatty-acid-deficient cell culture media (Engelhard *et al.*, 1976) alter liver plasma-membrane phospholipid fatty acid composition, membrane physical properties and the activity of membrane-bound enzymes. Considering the role of essential fatty acids in membranes, it is not surprising that these deficiencies alter membrane function and structure. More recently the principle that manipulation of dietary fat in nutritionally complete diets can change membrane lipid composition *in vivo* has also been demonstrated for other membranes (Clandinin, 1978; Tahin *et al.*, 1981; Wince & Rutledge, 1981; Innis & Clandinin, 1981*a*,*b*; Foot & Clandinin, 1982) and associated functions (Innis & Clandinin, 1981*c*).

It is possible that alterations of the structural lipid constituents of plasma membrane could modify adenylate cyclase activity through conformational interaction with the catalytic unit. In experiment 1, a diet effect was observed by feeding low-erucic acid oil at two levels of fat intake providing 20% and 40% of energy as fat. This difference in fat intake resulted in membrane lipid compositional changes and alteration in glucagon-stimulated adenylate cyclase activity. In this regard, it must be considered that the diet effect observed could be due in part to altered plasma glucagon concentrations resulting from different dietary carbohydrate loads. It is known that glucagon-stimulated adenylate cyclase activity can be influenced by changes in the number of glucagon-receptor-binding sites due to altered plasma glucagon levels. Chronic hyperglucagonaemia decreases glucagon binding and glucagon-stimulated adenylate cyclase activity in isolated hepatocytes (Bhathena *et al.*, 1978; Santos & Blazquez, 1982). The results of experiment 2, in which fat was fed at one level (providing 40% of energy as fat) and where no changes in plasma glucagon concentration would be expected, clearly indicate that dietary fat alters the activity of glucagon-stimulated adenylate cyclase through its membrane environment.

The membrane environment in reconstituted turkey erythrocytes influences both affinity and number of insulin receptors (Gould *et al.*, 1982). The role of membrane lipids in the coupling of receptor unit to the catalytic unit or, in conformational interactions, with the catalytic unit has been investigated through supplementation of the growth medium of isolated hepatocytes or tumour cells, with differing species of fatty acids (Poon *et al.*, 1981) and through removal and subsequent addition of phospholipids to membrane preparations (Rethy *et al.*, 1972; Rubakcava & Rodbell, 1973). Thus these studies suggest that diet may 'interact' with adenylate cyclase activation through the membrane lipid environment. The results of experiments 1 and 2 suggest that dietary fat influences the activity of a hormone-stimulated enzyme through dietary modulation of the membrane lipid environment. It is conceivable that a dose–response relationship between

the fatty acid composition of the fat being fed, the plasma-membrane phospholipid fatty acyl composition and the activity of glucagon-stimulated adenylate cyclase exists. Future studies should also consider that diet may also alter membrane phospholipid and cholesterol content. This relationship between diet fat and this receptor-mediated function has significant implications in whole-body metabolism, considering the widespread distribution of adenylate cyclase in body tissues, the involvement of glucagon in maintaining plasma glucose levels and the potential for modulation of analogous control mechanisms for metabolic regulation functioning at the plasma-membrane level throughout the body.

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